Accession No. 405399-06

DATA EVALUATION RECORD

1. **CHEMICAL:** Verbenone. Shaughnessey Number: 128986.

2. **TEST MATERIAL:** Verbenone; 100% active ingredient; a clear liquid.

3. **STUDY TYPE:** Acute Toxicity Test for Freshwater Fish. Species Tested: Rainbow trout (Salmo gairdneri).

4. **CITATION:** Surprenant, D.C. 1988. Static Acute Toxicity of Verbenone to Rainbow Trout (Salmo gairdneri). Submitted by Phero Tech, Inc., Vancouver, B.C., Canada. Study performed by Springborn Life Sciences, Inc., Wareham, MA. Laboratory Report No. 88-2-2632. EPA Accession No. 405399-06.

5. **REVIEWED BY:**

Michael L. Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: Michael L. Whitten
Date: 8-10-89

6. **APPROVED BY:**

Isabel C. Johnson, M.S.
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Isabel C. Johnson
Date: August 11, 1989

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature:
Date:

7. **CONCLUSIONS:** This study appears scientifically sound but does not fulfill the requirements for a 96-hour static acute toxicity study. Based upon nominal concentrations, the 96-hour LC50 of Verbenone to rainbow trout was 128 mg/L. This value classifies Verbenone as practically non-toxic to rainbow trout. The NOEC was determined to be 39 mg/L. Since the test chemical was partially insoluble, the actual concentrations to which the fish were exposed is unknown.

8. **RECOMMENDATIONS:** N/A

6 HRS

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Rainbow trout (Salmo gairdneri) were obtained from a commercial supplier in Montana. The fish were held in a 500 L fiberglass tank containing well water for a minimum of 14 days at 12-13°C. The fish were fed a dry commercial pelleted food, ad libitum, daily except during the 48 hours prior to testing. There was no mortality in the test fish population during the 48-hour period prior to testing. The fish used for the experiment had a mean wet weight of 0.45 g with a range of 0.30 to 0.66 g and a mean total length of 34 mm with a range of 32 to 41 mm.

B. **Test System:** The test was conducted in 18.9 L glass aquaria which contained 15 L of test solution. The test solution depth was 18.4 cm with a surface area of 819 cm^2. Test solutions were prepared by directly adding the appropriate quantity of Verbenone to 15 L of dilution water in each test aquarium and then mixed with a laboratory stirrer. A control aquarium was established and maintained under the same conditions as the test aquaria but contained no Verbenone.

The dilution water was soft water reconstituted from deionized water. The dilution water had a total hardness of 50 mg/L as $CaCO_3$, a total alkalinity of 30 mg/L as $CaCO_3$, a pH of 7.7, and a specific conductivity of 110 umhos/cm.

All test solution temperatures were controlled by a system designed to maintain temperatures at 12 $\pm$ 1°C. Test solutions were not aerated. A photoperiod of 16 hours of light and 8 hours of darkness was provided each day.

C. **Dosage:** 96-hour acute static test.

D. **Design:** A control and six nominal concentrations of 39, 65, 110, 180, 300, and 500 mg/L were tested. Ten fish selected impartially from the holding tank were placed in each test aquarium within 15 minutes after the test solutions had been prepared. The resulting test organism loading was 0.3 g of biomass per liter of test solution. Fish were not fed during the test.

All aquaria were observed at 0, 24, 48, 72, and 96 hours of exposure for mortality and abnormal effects. Dissolved oxygen, pH, and temperature were measured in all aquaria at each 24-hour interval.

E. **Statistics:** The LC50 was determined by a computerized calculation program (Peltier, 1985) for each 24-hour interval.

12. **REPORTED RESULTS:** The cumulative percent mortalities are presented in Table 1 (attached). At test initiation a film was present on the surface of all solutions containing Verbenone. Throughout the remainder of the exposure period a film of undissolved test material was present on the surface of all treatment solutions except at 39 mg/L.

The 24-, 48-, and 72-hour LC50 values were 220, 150, and 130 mg/L, respectively. The 96-hour LC50 was estimated by probit analysis to be 130 mg/L with a 95% confidence interval of 100 to 160 mg/L. Based on the results of this study, Verbenone is considered practically non-toxic to rainbow trout. The NOEC was 39 mg/L.

The dissolved oxygen concentrations and pH measured during the test are shown in Table 3 (attached). The dissolved oxygen concentration remained at or above 72% at 48 hours and 59% at 96 hours. The pH ranged from 7.3 to 7.6. The temperature ranged from 12°C to 13°C during the exposure period.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

The author presented no conclusions other than various remarks reported in Section 12, above.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report. The director stated that the study followed "...all pertinent EPA Good Laboratory Practice regulations except in the case of characterization and verification of the test substance identity."

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS**

A. **Test Procedure:** The test procedures were in accordance with recommended protocols with the following exceptions:

The SEP states that individual fish should weigh between 0.5 and 5 grams. Some fish used in this study weighed as low as 0.3 gram.

The SEP states that if test material solubility problems are encountered (i.e., a surface film of undisolved test material is present), chemical analyses are required to verify test exposure concentrations. Test concentrations were not measured in this study, although surface films were reported.

B. **Statistical Analysis:** Using EPA's Toxanal computer program, the LC50 was estimated by probit analysis to be 128 mg/L, with a 95 percent confidence interval of 103 to 156 mg/L, which is practically the same as reported by the author (LC50: 130 mg/L, 95% CI: 100-160 mg/L). The slope of the dose-response line was 8.9.

C. **Discussion/Results:** At test initiation a film was observed on the surface of all treatment solutions. Throughout the remainder of the exposure period, a film of undissolved test material was present on the surface of all but the lowest treatment solution. The partial insolubility of the test chemical indicates that the concentrations of toxicant were less than the nominal values.

The 96-hour no-observed-effect concentration (NOEC) was 39 mg/L, based on the loss of equilibrium and darkened pigmentation of fish in the 65 mg/L group. The 96-hour LC50 of 128 mg/L (based upon nominal concentrations) classifies Verbenone as practically non-toxic to rainbow trout (Salmo gairdneri).

The study appears to be scientifically valid, but since the test chemical was partially insoluble, the actual concentrations to which the fish were exposed are unknown.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental

(2) **Rationale:** Problems with solubility preclude a proper determination of toxicant concentrations.

(3) **Repairability:** Yes, if the registrant can show that the concentration of Verbenone was not seriously less than the nominal concentrations. An LC50 of greater than 100 mg/L would still classify Verbenone as practically non-toxic.

15. **COMPLETION OF ONE-LINER:** Yes; 8-3-89.

Table 1. Concentrations tested, corresponding cumulative mortalities and observations made during the 96 hour static exposure of rainbow trout (Salmo gairdneri) to Verbenone.

Nominal Concentration (mg/L)	Cumulative Mortality (%)			
	24-hour	48-hour	72-hour	96-hour
Control	0	0	0	0
39	0	0	0	0
65	0[ab]	0[abf]	0[af]	0[a]
110	0[acd]	20[ag]	30[ag]	30[a]
180	10[ae]	70[ah]	80[ai]	90[a]
300	100[a]	100[a]	100[a]	100[a]
500	100[a]	100[a]	100[a]	100[a]

[a] A film was present on the surface of the test solution.
[b] One of the surviving fish exhibited darkened pigmentation.
[c] One of the surviving fish exhibited an extended abdomen.
[d] Several of the surviving fish exhibited darkened pigmentation.
[e] All of the surviving fish exhibited a complete loss of equilibrium.
[f] Two of the surviving fish were on the bottom of the test solution.
[g] Several of the surviving fish exhibited a complete loss of equilibrium and darkened pigmentation.
[h] Several of the surviving fish exhibited a complete loss of equilibrium and were on the bottom of the test solution.
[i] Two of the surviving fish exhibited a complete loss of equilibrium and darkened pigmentation.

Table 3. pH and dissolved oxygen concentration measured during the 96-hour static exposure of rainbow trout (Salmo gairdneri) to Verbenone.

Nominal Concentration (mg/L)	0-hour	24-hour	48-hour	72-hour	96-hour
			pH		
Control	7.4	7.5	7.3	7.7	7.5
39	7.6	7.4	7.3	7.5	7.5
65	7.6	7.4	7.3	7.5	7.4
110	7.6	7.3	7.3	7.5	7.4
180	7.6	7.3	7.3	7.4	7.4
300	7.6	7.3	--[a]	---	---
500	7.6	7.4	---	---	---
			Dissolved Oxygen, mg/L (% Saturation)		
Control	9.6 (91)	8.8 (83)	8.8 (83)	8.8 (81)	7.6 (70)
39	9.6 (91)	8.8 (83)	8.4 (80)	8.0 (74)	7.0 (65)
65	9.6 (91)	8.2 (76)	8.2 (76)	7.9 (73)	6.4 (59)
110	9.8 (91)	7.9 (73)	8.0 (74)	7.8 (72)	6.4 (59)
180	9.8 (91)	8.8 (81)	7.8 (72)	7.4 (69)	6.4 (59)
300	9.8 (91)	8.8 (81)	---	---	---
500	9.8 (91)	9.2 (85)	---	---	---

[a]Measurement not required due to 100% mortality at previous 24 hour observation interval.

WHITTEN VERBENONE SALMO GAIRDNERI 08-02-89

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
500	10	10	100	9.765625E-02
300	10	10	100	9.765625E-02
180	10	9	90	1.074219
110	10	3	30	17.1875
65	10	0	0	9.765625E-02
39	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 65 AND 180 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 128.2827

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	.1144044	127.9145	95.81212	166.8144

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
11	.3518768	1	.9993244

SLOPE = 8.922888
95 PERCENT CONFIDENCE LIMITS = 3.629902 AND 14.21587

LC50 = 127.5951
95 PERCENT CONFIDENCE LIMITS = 103.4409 AND 156.1175

LC10 = 91.93972
95 PERCENT CONFIDENCE LIMITS = 52.97309 AND 110.8869

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Chemical No. 128986 Chemical Name Verbenone Chemical Class ____ Page) of 1

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Valid Study
14-Day Single Dose Oral LD_{50}		LD50 = . mg/kg (95% C.L.) Contr. Mort.(%)=		
Species ____		Slope= # Animals/Level= Age(Days)= Sex =		
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L.) Contr. Mort.(%)=		
Species ____		Slope= # Animals/Level= Age(Days)= Sex =		
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab ____		8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab ____		8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
48-Hour LC_{50}		LC50 = pp__ (95% C.L.) Contr. Mort.(%)= Sol. Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Temperature =		
Lab ____		48-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
96-Hour LC_{50}		LC50 = 128* ppm (95% C.L. 103-156) Con. Mor.(%)= 0 Sol. Con. Mor.(%)= N/A	M. WHITTEN 8-3-89	
Species Salmo gairdneri		Slope= 8.9 # Animals/Level= 10 Temp.= 12-13°C		SUPPLEMENTAL
Lab Springborn Life Sciences 100%		96-Hour Dose Level pp /(%Mortality) 39 (0), 65 (0), 110 (30), 180 (90), 300 (100), 500 (100)		
Acc. 405399-06		Comments: BASED ON NOMINAL CONCENTRATIONS		
96-Hour LC_{50}		LC50 = pp__ (95% C.L.) Con. Mort.(%)= Sol. Con. Mort.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab ____		96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		